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(57) Abstract

A method and composition for the solubilization of hydrophobic materials using a lysophospholipid. The method includes drying a composition comprising a hydrophobic material-solubilizing effective amount of phospholipid from organic solvent and hydrating the resulting film with an aqueous medium at either a pH of between about 8.5 and about 14.0, or at pH 7.0 followed by reduction of the temperature to less than 0°C.

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SOLUBILIZATION OF HYDROPHOBIC MATERIALS USING LYSOPHOSPHOLIPID

BACKGROUND OF THE INVENTION

The present invention is directed to the field of solubilization of hydrophobic materials. More particularly, the invention describes the use of certain lysophospholipids as solubilizers and non-toxic delivery vehicles.

The solubilization of hydrophobic materials, particularly bioactive materials, is typically achieved by the use of surfactants such as sodium deoxycholate or propylene glycol. Such surfactants, due to their detergent properties, are biologically incompatible and toxic due to their lytic effects on cells. It would, therefore, be desirable to employ a naturally occurring substance without toxic properties as a surfactant for pharmaceutical use.

Lysophospholipids have heretofore been improbable candidates for pharmaceutical excipients due to their lytic effects on cells. We describe a method and compositions for a lysophosphatide, specifically lysophosphatidylethanolamine, alone and in combination with an unsaturated phospholipid for solubilizing hydrophobic materials.

Peterson et al., Arch Biochem Biophys, 179, 218-228 (1977), observed lysophosphatidylethanolamine's (LPE) properties, as an ATPase inhibitor in biomembranes; these effects probably due to LPE intrusion into the membrane around the enzyme resulting in a less fluid lipid environment. Lysophospholipid suspensions were mixed with fractions of sarcoplasmic reticulum and changes in ATPase activity were recorded using a spectrophotometric assay.

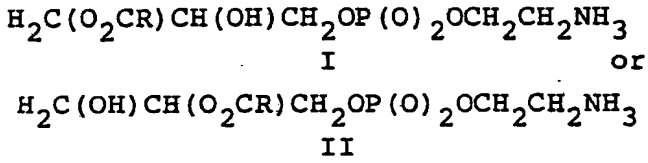
LPE, however, unlike lysophosphatidylcholine, never solubilized biomembrane at any concentration.

The present invention exploits the pH and temperature dependent phase transitions of lysophosphatidylethanolamines to result in micellar solubilization of hydrophobic materials and delivery of a non-toxic product.

SUMMARY OF THE INVENTION

The present invention describes solubilization of hydrophobic substances using LPE alone or LPE in combination with an unsaturated phospholipid.

The LPE's of the present invention have the formula



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or

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wherein R is a hydrocarbon chain having between about 11 and 21 carbon atoms and between about 0 and 6 double bonds, preferably 13-19 carbon atoms, and more preferably 15-17 carbon atoms and 1-3 double bonds. A preferred LPE is that wherein RCO_2 is 1-oleoyl, as in formula I above, where the oleoyl group is bound to the 1 carbon atom.

A phospholipid is a glycerol molecule having one hydroxyl esterified to phosphoric acid which is further esterified to an alcohol component; and the other glycerol hydroxyls are esterfied to carboxylate fatty acid chains. The unsaturated phospholipids used in the present invention contain at least one fatty acid chain of between about 12 and 22 carbon atoms and about 1 to 6 double bonds, preferably about 16 to 20 carbon atoms and about 1 to 3 double bonds. The second fatty acid chain contains about 12 to 22 carbon atoms and about 0 to 6 double bonds, preferably

about 14 to 18 carbon atoms and about 0 to 2 double bonds, more preferably 0 or 1 double bonds. Suitable phospholipids include, but are not limited to, derivatives of phosphatidylserine, phosphatidylethanolamine, phosphatidylinositol or phosphatidylcholine. Preferred unsaturated phospholipids include egg phosphatidylcholine, soy phosphatidylcholine or dioleoylphosphatidylcholine.

The lysophospholipid, LPE, alone or in combination with a phospholipid, is mixed with the hydrophobic material in an aqueous medium at a temperature of between about 1°C and about 90°C, and at pH between about 8.5 and 14.0, preferably about 25°C and about pH 8.5. Alternatively, a more highly unsaturated LPE may be used; in this case, the hydrophobic material may be mixed with the lipid and aqueous medium at a temperature of between about 0°C and about 90°C, preferably about 25°C and at about pH 7.0. The temperature of the suspension is then reduced to below 0°C. The hydrophobic material can comprise bioactive agents including, but not limited to, drugs, hormones, proteins, dyes, vitamins or imaging agents. The aqueous medium can comprise a buffer system such as borate or N-2-hydroxyethyl piperazine-N'-2-ethane sulfonic acid (HEPES). The resulting suspension may be passed under pressure through a filter system such as stacked polycarbonate filters and may be sonicated to further disperse the hydrophobic material.

BRIEF DESCRIPTION OF THE DRAWING

FIGURE 1 are 81MHz ^{31}P-NMR spectra of aqueous dispersions of 1-oleoyl lysophosphatidylethanolamine (LOPE, or sn-1-18:1$_{cis}$-PE) at pH 7.0 between -20°C and 90°C.

FIGURE 2 are ^{31}P-NMR spectra showing the effect of pH variation on the polymorphic phase behavior of LOPE.

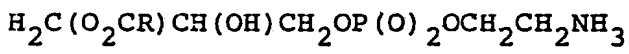
FIGURE 3 are ^{31}P-NMR spectra showing the effect of temperature variation on the polymorphic phase behavior of (A) sn-1-18:2_{cis}-PE and (B) sn-1-18:3_{cis}-PE.

FIGURE 4 is a graph depicting the hemolytic properties of various phospholipids.

FIGURE 5 is an expanded scale of hemolytic properties of various phospholipids.

DETAILED DESCRIPTION OF THE INVENTION

1-oleoyl lysophosphatidylethanolamine may be expressed as



wherein R is the oleoyl group attached to the carbon in the 1-position, as labeled [1], thus 1-oleoyl. The (OH) group is located on the [2] carbon. This lipid may be further expressed as sn-1-18:1_{cis}-PE, denoting the 18 carbon composition of the oleoyl group, followed by a number denoting the number of double bonds, in ths case 1 double bond in the cis configuration. As a further illustration of the nomenclature, for example, more highly unsaturated LPE's, wherein R is in the 1-position and has 2 or 3 double bonds and 17 carbon atoms; are expressed as sn-1-18:2_{cis}-PE and sn-1-18:3_{cis}-PE, respectively. The carboxylate carbon atom is the 18th carbon atom.

We have found that 1-oleoyl lysophosphatidyl-ethanolamine (LOPE, or sn-1-18:1_{cis}-PE) exhibits a lamellar phase at physiological pH rather than the micellar arrangement of other lysophospholipids. LOPE, however,

exhibits a micellar state at higher pH (i.e., at about 8.5 or higher) which promotes micellar solubilization of hydrophobic substances under such conditions. This polymorphic phase behavior from micellar to bilayer states is substantiated by: (a) ^{31}P-NMR spectra, which correspond to a lamellar configuration at pH 7 at temperatures of -20°C to 90°C, in contrast to lysophosphatidylcholine which is micellar (Fig. 1); (b) x-ray diffraction patterns of LOPE, in which the x-ray scatter forms equidistantly spaced rings, indicative of a lamellar organization; (c) freeze fracture micrographs which show the unilamellar nature of LUVETS produced at pH 7.0; and (d) ^{31}P-NMR spectra that show isotropic motional averaging at pH 9.0, indicative of a micellar structure (Fig. 2). This polymorphic phase behavior allows micellar solubilization of a hydrophobic material at a pH of about 8.5, and bilayer formation at lower pH.

We have also found that lysophosphatidylethanolamines having 2 or 3 double bonds such as sn-1-18:2_{cis}-PE and sn-1-18:3_{cis}-PE, respectively, exhibit polymorphic phase behavior in response to temperature variation. Below 0°C, both lipids give rise to ^{31}P-NMR spectra indicative of overall lamellar organization indicated by a low field shoulder followed by a peak (Fig. 3). Both sn-1-18:2_{cis}-PE and sn-1-18:3_{cis}-PE show a hexagonal structure at 0-1°C. However, at 10-20°C and above, both lipids are in the (inverted) micelle or H_I state.

In the present invention, a lipid film and hydrophobic material are mixed in an aqueous medium resulting in solubilization of the hydrophobic material. This solubilization may be achieved by several methods. In the case where LOPE is the solubilizing lipid used, the hydrophobic material is combined with LOPE in an amount sufficient to solubilize it, and both dried to a film in a

receptacle, from an organic solvent. Suitable organic solvents are those with a variety of polarities and dielectric properties, including chloroform, acetone, methylene chloride, diethyl and petroleum ethers, and mixtures of chloroform and methanol. All of the above-mentioned solvents will dissolve the phospholipids. The dry film is then hydrated with an aqueous medium at pH of between about 8.2 and about 14.0. Alternatively, a dry film of LOPE may be hydrated with an aqueous medium at pH of between about 8.2 and 14.0, followed by addition of the hydrophobic material. Lastly, an aqueous medium at pH of between about 8.2 and about 14.0 containing a hydrophobic material may be used to hydrate a dry film of LOPE. The hydrophobic material may be a bioactive agent.

In the preferred embodiment, lipid and hydrophobic material are dried under vacuum from chloroform to a thin film. The dried film is then hydrated with an aqueous buffer such as borate, HEPES, or potassium glutarate (KGlu) at pH 8.5-14.0; most preferably about pH 8.5. In general, in the 8.5-14.0 pH range, lysophosphatidylethanolamine assumes its micellar state. At pH of about 8.0 and lower, LOPE is in a lamellar state. The hydrophobic material is rehydrated with the lipid in an aqueous medium with agitation and/or vortical mixing. The concentration of hydrophobic material can preferably range from about 5-25 mg/ml of buffer. The LOPE dispersion was held at about 4^{o}C for 2-3 hours to favor micellization. This dispersion is optionally then subjected to up to about 10 repeated extrusions at pressures of about 700 psi using an extrusion apparatus; this method and "LUVET" apparatus described in a copending application, Serial No. 622,690, filed June 20, 1984, Pieter R. Cullis et.al., "Extrusion Technique for Producing Unilamellar Vesicles", relevant portions of which are incorporated herein by reference. The samples were held at about 20-30^{o}C, preferably about 25^{o}C for 16-18 hours

to confirm complete solubilization, evidenced by lack of precipitation. The solubilized product can be used as an injectable product administered, for example, intravenously, intramuscularly, or subcutaneously, in a subject including mammals such as humans. The product is best used in the form of a sterile aqueous solution which may contain other solutes, for example, enough salts or glucose to make the solution isotonic.

In cases where sn-1-18:2_{cis}-PE or sn-1-18:3_{cis}-PE's are the solubilizing lipids used, the hydrophobic material may be combined with the lipid in an amount sufficient to solubilize it, and both dried to a film in a receptacle, from organic solvent. The dry film may then be hydrated with an aqueous medium at pH between about 6.0 and 8.0 and held at a temperature from about -90°C to 0°C, preferably about -20°C, which favors the lamellar phase of the lipid. Alternatively, a dry lipid film may be hydrated with an aqueous medium at pH of between about 6.0 and 8.0 followed by addition of or combined with the hydrophobic material. The solubilized product may be stored at this reduced temperature, in lamellar phase lipid for purposes of enhancing shelf life.

MATERIALS AND METHODS

Dioleoyl phosphatidylethanolamine (DOPE) was prepared from dioleoyl PC according to established procedures, Confurius, P. et al., Biophys. Biochem. Acta., 488, 36-42 (1977). Lysophosphatidylethanolamine was prepared according to the following protocol: 500 mg DOPE was dissolved in 50 ml anhydrous diethyl ether to which was added 10 ml 0.5M Tris/HCl buffer (pH 7.4), 10 ml of 2.5 mM $CaCl_2$ and 100 mg of Crotalus adamanteus venom (Sigma Chemical Co., St. Louis, MO). The reaction vessel was flushed with nitrogen, sealed, covered with aluminum foil, and stirred vigorously at room

temperature. The mixture was rotoevaporated under reduced pressure to remove the diethyl ether and the aqueous phase extracted with chloroform methanol 2:1 v/v followed by chloroform. The preparation was purified by liquid chromatography using a Waters Prep 500 LC unit. Purity was further verified by H-NMR.

The present invention is exemplified by the following Examples, but the scope of the invention is not limited thereto.

EXAMPLE 1

Fifty μmol of LOPE combined with 5 mg of 21-acetoxy-pregnenolone (Sigma Chemical Co., St. Louis, MO) was rotoevaporated to a dried film from chloroform onto the inner sides of a test tube. One ml of borate buffer (100 mM $NaHCO_3$,50 mM borate), pH 8.5, was added to the tube and the lipid dispersed by vortexing. After dispersion by vortexing, the preparations were left to stand at 4°C for 2-3 hours. The dispersions were then transferred into the pressure chamber of the LUVET apparatus, equipped with two stacked 100 nm polycarbonate filters. Positive pressure was applied to the chamber by way of a standard nitrogen cylinder at 500 psi. The pressure was adjusted within the 200-900 psi range to allow complete extrusion of the sample without the membrane filter clogging. Application of pressure results in the extrusion of the sample through the filters. Each sample was extruded a total of ten times.

The above procedures were repeated using 10, 15, 20, and 25 mg of 21-acetoxypregnenolone.

The samples were left at 25°C for 16-18 hours, after which time all samples appeared clear; viscosity increasing with increasing 21-acetoxypregnenolone content.

EXAMPLE 2

The procedures and materials of Example 1 were employed using LOPE and DOPC in a 1:1 molar ratio to solubilize 10 mg of 21-acetoxypregnenolone. Following the LUVET treatment, the solution was allowed to stand for 16-18 hours at 25°C, and a further LUVET filtration employing a 50 nm filter system at a pressure of 200 psi was performed. Following this treatment, no particulate material was observed on the filter, and the preparation appeared translucent. Attempts to solubilize 20 mg of steroid in 1.0 ml of buffer were unsuccessful.

EXAMPLE 3

Hemolytic properties of 1-oleoyl lysophosphatidylethanolamine (LOPE) were tested at both pH 7.0 and 8.5, and compared to those of 1-oleoyl lysophosphatidylcholine (LOPC) and dipalmitoylphosphatidylethanolamine (DPPE). LOPE, LOPC, and DPPE stock solutions were made at 12-13 mg/ml in 50 mM borate buffer at pH 7.0 and 8.5. Following the additions of 0.1 ml volumes of stock solution of one of these compounds to 1.0 ml aliquots of heparinized whole blood, hemolytic activities were assayed by spectrophotometric measurement of the centrifuged supernatants at 550 nm.

FIG. 4 shows the reduced hemolytic activity of LOPE at both pH 7.0 and 8.5 as compared to LOPC, when added to whole blood. LOPC produced the greatest amount of red blood cell lysis, liberating the most hemoglobin. High concentrations of LOPE produced lysis as compared to DPPE, which caused minimal lysis (FIG. 5). Control experiments with borate buffer alone at both pH 7.0 and 8.5 produced no lysis.

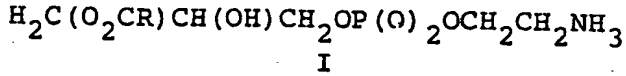
EXAMPLE 4

Fifty µmol of sn-1-18:2_{cis}-PE combined with 5 mg of 21-acetoxypregnenolone is rotoevaporated to a dry film from chloroform onto the inner sides of a test tube. One ml of borate buffer, pH 7.0, is added to the tube and the lipid dispersed by vortical mixing. After dispersion, the suspension is cooled to -20°C, and transferred into the pressure chamber of the LUVET and extruded using the procedures of Example 1.

CLAIMS

1. A method of solubilizing a hydrophobic material comprising the steps of:

(a) removing an organic solvent in which a composition comprising a hydrophobic-material- solubilizing effective amount of a lysophosphatidylethanolamine of the formula:



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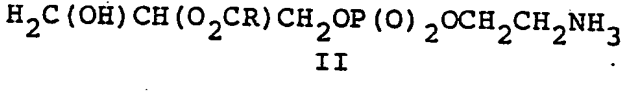

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is dissolved to obtain a film; and

(b) hydrating the film with an aqueous medium at pH of between about 8.5 and about 14.0;

wherein R is a hydrocarbon chain having between about 11 and 21 carbon atoms and 1 double bond.

2. The method of Claim 1 wherein the composition comprises the hydrophobic material.

3. The method according to Claim 2 wherein the hydrophobic material is a bioactive agent.

4. The method of Claim 1 wherein step (b) is followed by the step of adding the hydrophobic material.

5. The method according to Claim 4 wherein the hydrophobic material is a bioactive agent.

6. The method of Claim 1 wherein the hydrophobic material is combined with the aqueous medium prior to hydrating the film in step (b).

7. The method according to Claim 6 wherein the hydrophobic material is a bioactive agent.

8. The method according to Claim 1 wherein the aqueous medium is at pH of about 8.5.

9. The method according to Claim 1 wherein the lysophosphatidyl- ethanolamine has formula I.

10. The method according to Claim 9 wherein R has between about 13 to 19 carbon atoms.

11. The method according to Claim 9 wherein R has between about 15 to 17 carbon atoms, and 1 double bond.

12. The method according to Claim 9 wherein RCO_2 is 1-oleoyl.

13. The method according to Claim 1 wherein RCO_2 is 1-oleoyl.

14. The method according to Claim 1 wherein the hydrophobic material is a bioactive agent.

15. The method according to Claim 1 wherein the aqueous medium comprises aqueous buffer.

16. The method according to Claim 1 comprising the additional step of: (c) filtering the product of step (b).

17. The method according to Claim 16 comprising the additional step of administering parenterally the product of step (b).

18. The method according to Claim 1 wherein the composition additionally comprises an unsaturated phospholipid.

19. The method according to Claim 18 wherein the unsaturated phospholipid comprises at least one unsaturated fatty acid chain of between about 12 and 22 carbon atoms and 1 to 6 double bonds.

20. The method according to Claim 19 wherein the unsaturated phospholipid has between about 16 to 20 carbon atoms and 1 to 3 double bonds.

21. The method according to Claim 19 wherein the second fatty acid chain of the unsaturated phospholipid has between about 12 and 22 carbon atoms and 0 to 6 double bonds.

22. The method according to Claim 21 wherein the second fatty acid chain of the unsaturated phospholipid has between about 16 and 20 carbon atoms and 1 to 3 double bonds.

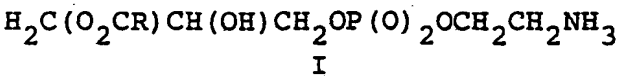
23. The method according to Claim 19 wherein the unsaturated phospholipid is selected from the group consisting of egg phosphatidylcholine, soy phosphatidylcholine, and dioleoylphosphatidylcholine.

24. The method according to Claim 19 comprising the additional step of filtering the product of step (b).

25. The method according to Claim 24 comprising the additional step of administering parenterally to a mammal the product of the filtering step.

26. A method of solubilizing a hydrophobic material comprising the steps of:

(a) removing an organic solvent in which a composition comprising a hydrophobic-material-solubilizing effective amount of lysophosphatidylethanolamine of the formula:



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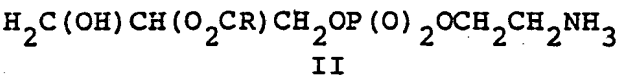

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is dissolved to obtain a film;

(b) hydrating the film with an aqueous medium at pH of between about 6.0 and about 8.0; and

(c) cooling the dispersed lipid in aqueous medium to a temperature below about 0°C;

wherein R is a hydrocarbon chain having between about 11 and 21 carbon atoms and about 2 to 6 double bonds.

27. The method of Claim 26 wherein the composition comprises the hydrophobic material.

28. The method according to Claim 27 wherein the hydrophobic material is a bioactive agent.

29. The method of Claim 26 wherein step (b) is followed by the step of adding the hydrophobic material.

30. The method according to Claim 29 wherein the hydrophobic material is a bioactive agent.

31. The method of Claim 28 wherein a hydrophobic material is combined with the aqueous medium prior to hydrating the film in step (b).

32. The method according to Claim 31 wherein the hydrophobic material is a bioactive agent.

33. The method according to Claim 26 wherein the aqueous medium is at pH of about 7.0.

34. The method according to Claim 26 wherein the lysophosphatidyl- ethanolamine has formula I.

35. The method according to Claim 34 wherein R has between about 13 to 19 carbon atoms.

36. The method according to Claim 34 wherein R has between about 15 to 17 carbon atoms, and 2 or 3 double bonds.

37. The method according to Claim 34 wherein RCO_2 is 1-oleoyl.

38. The method according to Claim 26 wherein RCO_2 is 1-oleoyl.

39. The method according to Claim 26 wherein the aqueous medium comprises aqueous buffer.

40. The method according to Claim 26 comprising the additional step of: (d) filtering the product of step (c).

41. The method according to Claim 26 wherein the composition additionally comprises an unsaturated phospholipid.

42. The method according to Claim 41 wherein the unsaturated phospholipid comprises at least one unsaturated fatty acid chain of between about 12 and 22 carbon atoms and 1 to 6 double bonds.

43. The method according to Claim 42 wherein the unsaturated phospholipid has between about 16 to 20 carbon atoms and 1 to 3 double bonds.

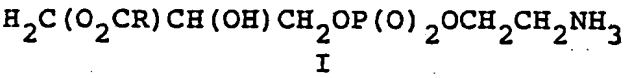
44. The method according to Claim 43 wherein the second fatty acid chain of the unsaturated phospholipid has between about 12 and 22 carbon atoms and 0 to 6 double bonds.

45. The method according to Claim 44 wherein the second fatty acid chain of the unsaturated phospholipid has between about 16 and 20 carbon atoms and 1 to 3 double bonds.

46. The method according to Claim 45 wherein the unsaturated phospholipid is selected from the group consisting of egg phosphatidylcholine, soy phosphatidylcholine, and dioleoylphosphatidylcholine.

47. The method according to Claim 41 comprising the additional step of filtering the product of step (c).

48. A composition comprising an aqueous solution at between about pH 8.5 to 14.0 of a hydrophobic material and a lysophospholipid of the formula:



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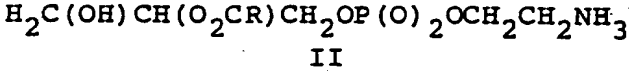

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wherein R is a hydrocarbon chain having between about 11 and 21 carbon atoms and 1 double bond.

49. The composition according to Claim 48 wherein the aqueous solution is at a pH of about 8.5.

50. The composition of Claim 48 wherein the lysophosphatidylethanolamine has formula I.

51. The composition of Claim 48 wherein R has between about 13 to 19 carbon atoms.

52. The composition of Claim 48 wherein R has between about 15 and 17 carbon atoms.

53. The composition of Claim 48 wherein R has 1 or 2 double bonds.

54. The composition of Claim 48 where RCO_2 is 1-oleoyl.

55. The composition of Claim 48 wherein the hydrophobic material is a bioactive agent.

56. The composition of Claim 48 wherein the aqueous solution comprises aqueous buffer.

57. The composition according to Claim 48 additionally comprising an unsaturated phospholipid.

58. The composition of Claim 57 wherein the unsaturated phospholipid comprises at least one unsaturated fatty acid chain of between about 12 and 22 carbon atoms and 1 to 6 double bonds.

59. The composition of Claim 57 wherein the second fatty acid chain of the unsaturated phospholipid comprises between about 12 and 22 carbon atoms and 0 to 6 double bonds.

60. The composition of Claim 57 wherein the unsaturated phospholipid has between about 16 to 20 carbon atoms and 1 to 3 double bonds.

61. The composition of Claim 57 wherein the unsaturated phospholipid is selected from the group consisting of egg phosphotidylcholine, soy phosphatidylcholine, and dioleoylphosphatidylcholine.

62. A composition comprising an aqueous solution at between about pH 6.0 to 8.0 of a hydrophobic material and a lysophospholipid of the formula:

$$H_2C(O_2CR)CH(OH)CH_2OP(O)_2OCH_2CH_2NH_3$$

I

or

$$H_2C(OH)CH(O_2CR)CH_2OP(O)_2OCH_2CH_2NH_3$$

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wherein R is a hydrocarbon chain having between about 11 and 21 carbon atoms and about 2 to 6 double bonds, and wherein the temperature of the composition is between about -20°C and about 0°C.

63. The composition according to Claim 62 wherein the aqueous solution is at a pH of about 7.0.

64. The composition of Claim 62 wherein the lysophosphatidylethanol- amine has formula I.

65. The composition of Claim 62 wherein R has between about 13 to 19 carbon atoms.

66. The composition of Claim 62 wherein R has between about 15 and 17 carbon atoms, and 2 or 3 double bonds.

67. The composition of Claim 62 where RCO_2 is 1-oleoyl.

68. The composition of Claim 62 wherein the hydrophobic material is a bioactive agent.

69. The composition of Claim 62 wherein the aqueous solution comprises aqueous buffer.

70. The composition according to Claim 70 additionally comprising an unsaturated phospholipid.

71. The composition of Claim 70 wherein the unsaturated phospholipid comprises at least one unsaturated fatty acid chain of between about 12 and 22 carbon atoms and 1 to 6 double bonds.

72. The composition of Claim 70 wherein the second fatty acid chain of the unsaturated phospholipid comprises between about 12 and 22 carbon atoms and 0 to 6 double bonds.

73. The composition of Claim 70 wherein the unsaturated phospholipid has between about 16 to 20 carbon atoms and 1 to 3 double bonds.

74. The composition of Claim 70 wherein the unsaturated phospholipid is selected from the group consisting of egg phosphotidylcholine, soy phosphatidylcholine, and dioleoylphosphatidylcholine.

FIG. 1

^{31}P-NMR SPECTRA OF LOPE AT $_p$H 7

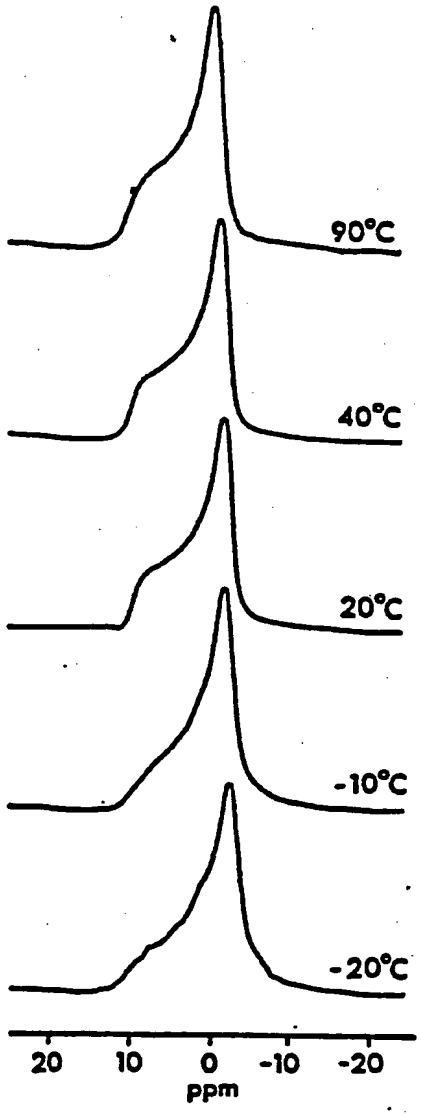


FIG. 2

^{31}P-NMR SPECTRA OF pH DEPENDENT POLYMORPHIC PHASE BEHAVIOR OF LOPE

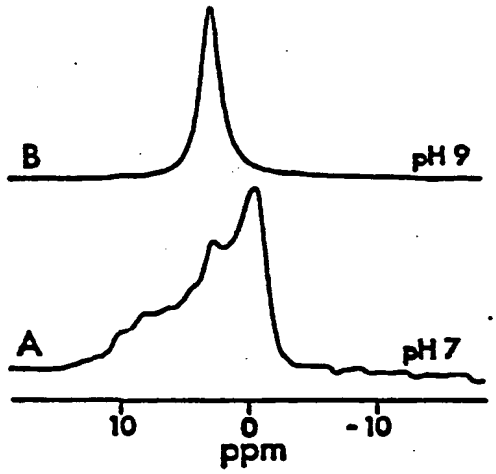
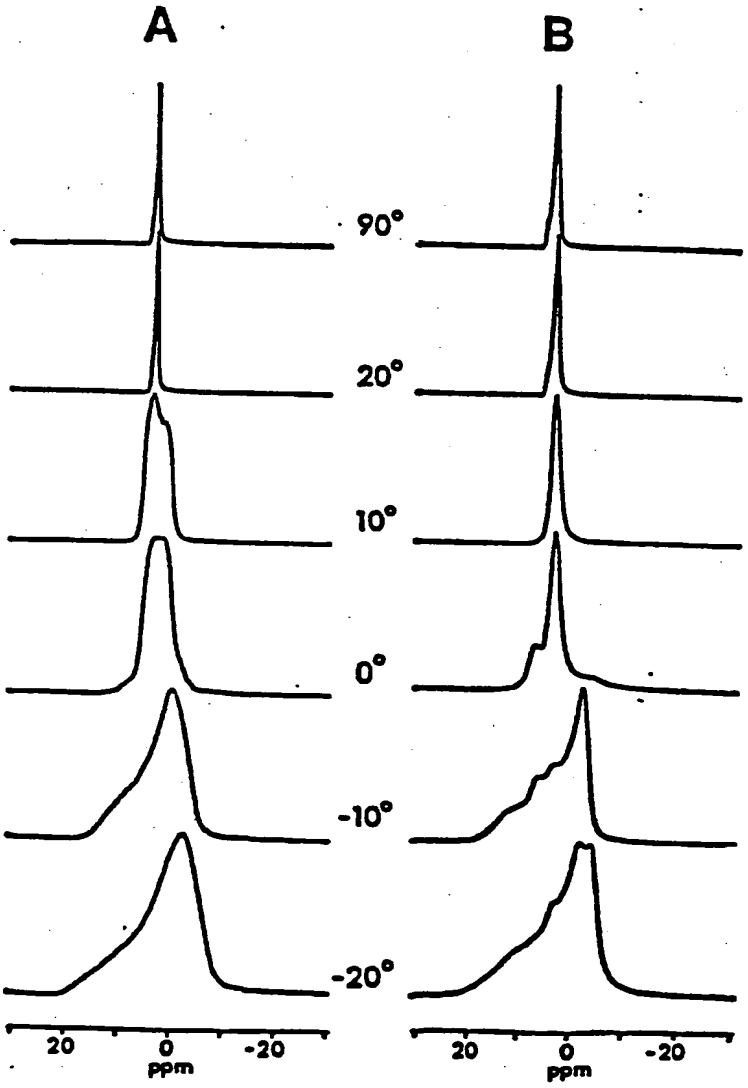


FIG. 3

^{31}P-NMR SPECTRA OF TEMPERATURE-DEPENDENT POLYMORPHIC PHASE BEHAVIOR OF sn-1-18:2$_{cis}$-PE AND sn-1-18:3$_{cis}$-PE



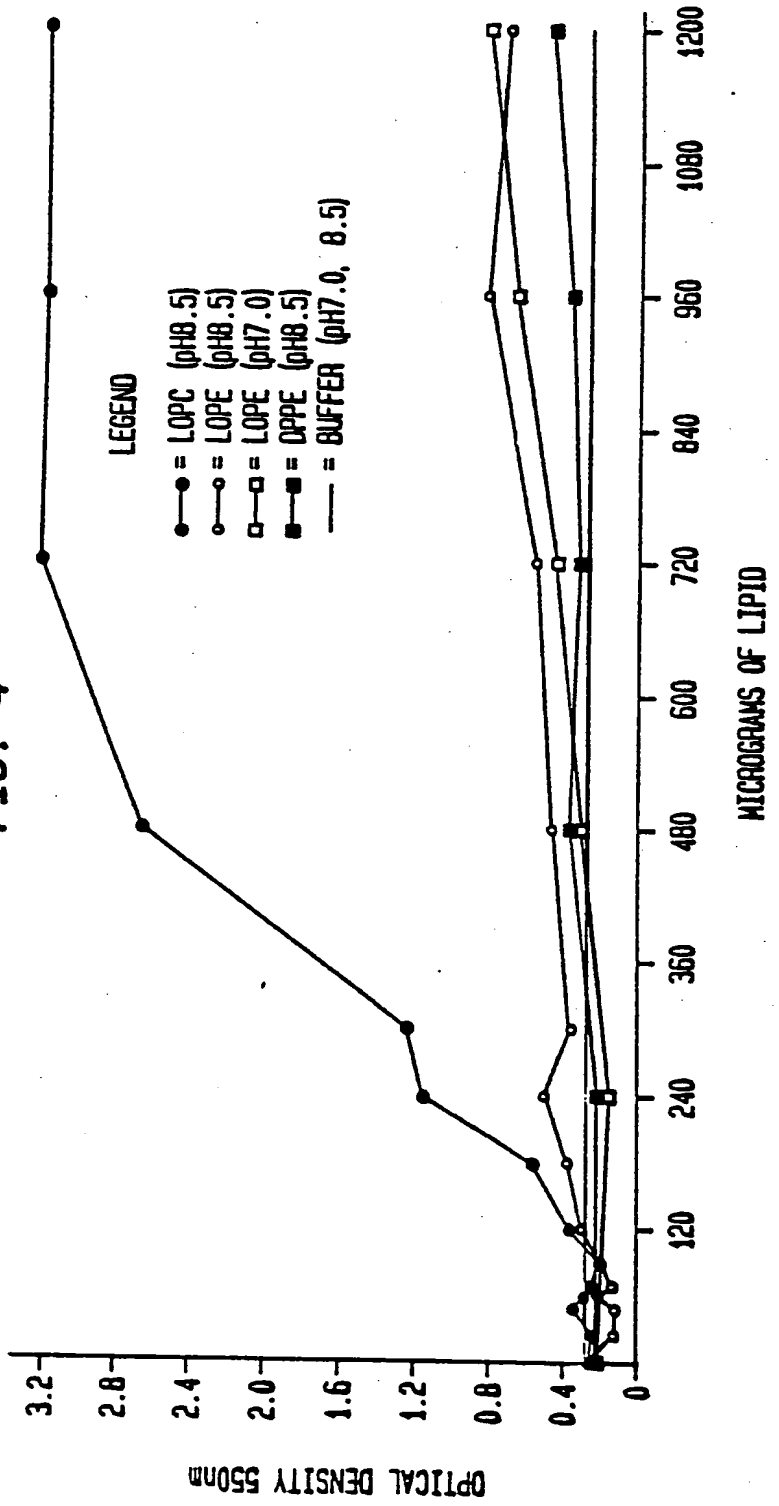

OPTICAL DENSITY 550nm
3.2
2.8
2.4
2.0
1.6
1.2
0.8
0.4
0
120
240
360
480
600
720
840
960
1080
1200
MICROGRAMS OF LIPID
LEGEND
= LOPC (pH8.5)
= LOPE (pH8.5)
= LOPE (pH7.0)
= DPPE (pH8.5)
= BUFFER (pH7.0, 8.5)

FIG. 4

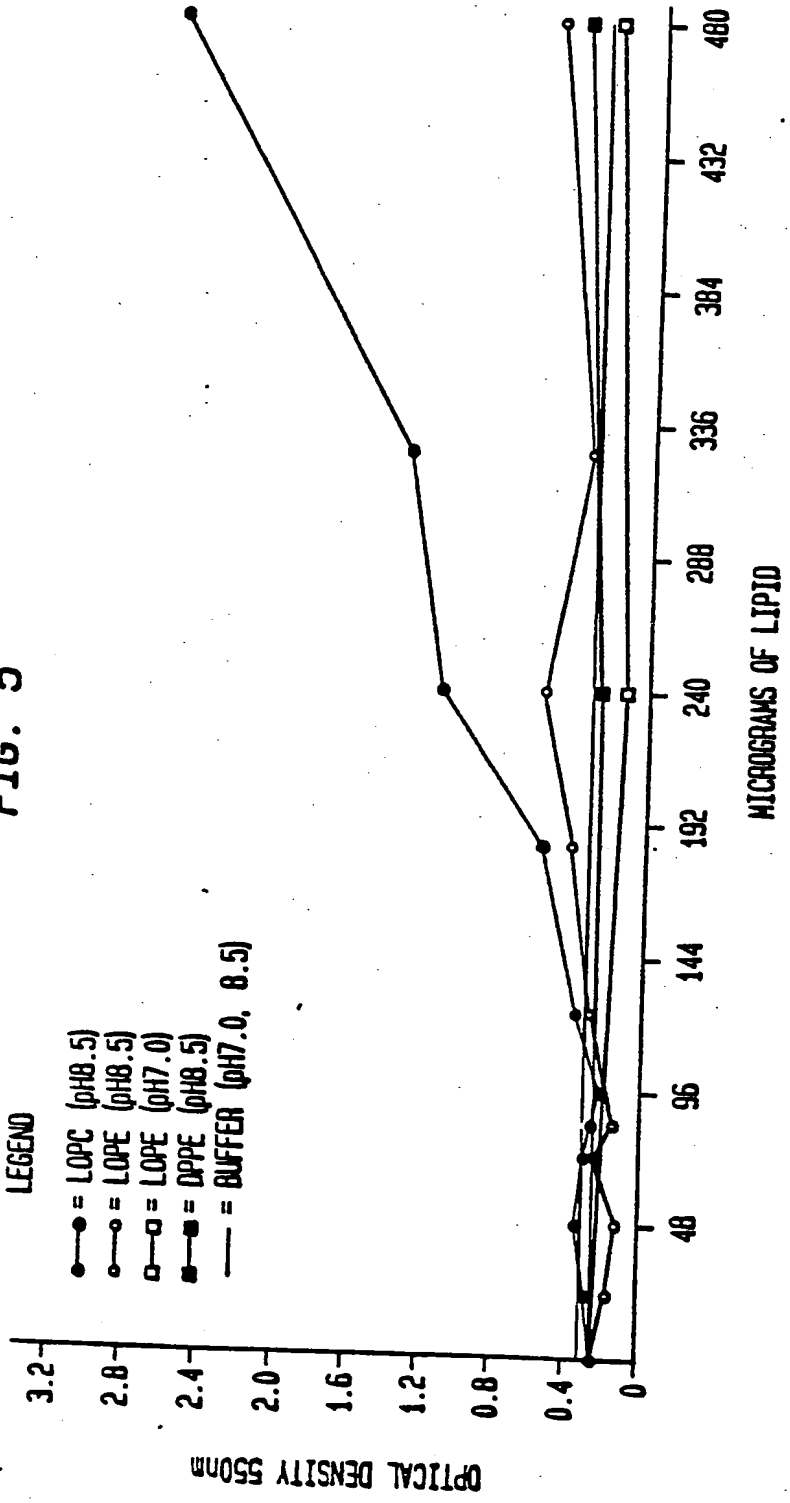

OPTICAL DENSITY 550nm
3.2
2.8
2.4
2.0
1.6
1.2
0.8
0.4
0
48
96
144
192
240
288
336
384
432
480
MICROGRAMS OF LIPID
LEGEND
= LOPC (pH8.5)
= LOPE (pH8.5)
= LOPE (pH7.0)
= DPPE (pH8.5)
= BUFFER (pH7.0, 8.5)

FIG. 5

INTERNATIONAL SEARCH REPORT

International Application No PCT/US87/00143

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC

INT. CL(4): A61K 31/685
U.S. CL : 514/78

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
U.S.	514/78

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [5]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
Y	US,A, 4,239,754 (SACHE ET AL) 16 December 1980 (16.12.80) See col. 3, lines 6-66.	1-74
T	US,A, 4,622,219 (HAYNES) 11 November 1986 (11.11.86) See col. 3, line 66 - Col. 8, line 9.	1-74
A	US,A, 4,263,286 (NAKAJIMA ET AL.) 21 April 1981 (21.04.81) See col. 1, line 63 - Col. 4, line 2.	1-74
A	US,A, 3,752,886 (MUNDER ET AL.) 14 August 1973 (14.08.73) See entire document.	1-74

* Special categories of cited documents: [13]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search [2]	Date of Mailing of this International Search Report [2]
07 May 1987	15 MAY 1987
International Searching Authority [1]	**Signature of Authorized Officer [20]**
ISA/US	Joseph Lipovsky

Form PCT/ISA/210 (second sheet) (May 1986)